Keys to the Identification of Plant remains in Animal droppings

BY

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(*With three plates*)

INTRODUCTION

This work was undertaken to assist the Bombay Natural History Society in their long term project on Ecological Research in Gir Forest in collaboration with Smithsonian Institution and Yale University, School of Forestry and financed by Smithsonian Foreign Currency Grant No. SFG-0-1894.

The help of this laboratory was required in this project to prepare and make available to the investigators, a diagnostic ' key ' using the microscopic characters of the plant debris present in the droppings of the wild animals at the Gir forest so that it could be used to ascertain the diet plants of these animals.

The work therefore involved the preparation of the supplied samples of faecal pellets and other allied materials in a form that would facilitate the microscopic identification of the plant debris present in them and the preparation of ' keys ' for the identification of their botanical source. The work was begun in July 1971 and completed by the middle of December.

MATERIALS

The following materials were supplied for the work through Mr. Berwick from Gir :

1. About 80 samples of plants of known identity, in dry state, consisting of twigs, and leaves and a few fruits in a few cases to serve as reference material.
2. About 90 samples of faecal pellets from a group of animals fed under control with known plant materials, to serve as reference material, and a list of about 20 plants that were the main diet of the animals.

3. About 20 samples from rumen of wild animals killed or dead, to serve as reference material.

4. About 140 samples of faecal pellets, being the droppings of wild animals like four-horned antelope, nilgai, sambar, chinkara, chital and hare.

Methods

A. **Preparation of materials :**

(1) *Reference slides from known plant samples :*

Permanent slides were prepared for study from the reference materials. Since the plant materials were said to be authoritatively identified, no identification was done at this laboratory. The samples were processed in the following manner :

A few bits of leaves, twigs and fruits (where present) were taken from each sample. These were shredded coarsely and placed in a test tube. Chloral hydrate aqueous solution (50 g. in 20 ml) was added to the material in the test tube, (about 2 or 3 ml). The tube was heated in a water bath (water at boiling point) for a minute or two. Highly coloured materials took a second boiling with fresh quantities of chloral hydrate. The tube was allowed to cool, the liquid drained off, washed repeatedly in distilled water, dehydrated in alcohol, passed through grades of alcohol : xylol mixtures with the latter in increasing proportion, in successive mixtures, (alcohol : xylol ; 3 : 1, 1 : 1, 1 : 3) and finally, in pure xylol. The mounting was done in canada balsam or aroclor 5442, keeping the slides over a warming plate.

(2) *Reference slides from droppings of control group of animals :*

The faecal pellets were ' sampled ' according to a method described later. The pellets were triturated with water, boiled for a short time, allowed to cool, the supernatant with scum poured off, sediment washed twice or thrice in fresh quantities of water to clarify as much as possible prior to chloral hydrate treatment. They were then subjected to the same processing as explained above in chloral hydrate and mounted.

(3) *Slides from ' wild ' animal droppings :*

A *starting sample* of whole pellets (which is to be powdered later), was prepared in the following manner: The '*final sample*' in the form of powder which is clarified and mounted for observation, is taken in a manner indicated later,

B. Sampling Procedure adopted :

(1) *Pellets exceeding 25 in number :*

The sample is put in a tray, shaken and tossed about several times before spreading them on the tray ; it is first halved and then quartered. Two opposite quarters are combined ; one such combined portion is rejected. The remaining combined portion is shaken and tossed in the same manner, spread again, halved and quartered again ; opposite quarters are combined ; one combined portion is rejected, as in the first quartering and the other retained. The halving and quartering and combining is continued till there are 3 or 4 pellets in each quarter ; the opposite quarters are combined now to get two portions of 6 or 8 pellets each ; one of this is rejected and the other retained ; this is the *starting sample.*

(2) *For samples less than 25 but over 5 :*

The sample is put in a tray, rolled and tossed in all directions till the pellets are thoroughly mixed ; the sample is spread and individual pellets picked up from various portions till about six or eight are obtained ; This is the *starting sample.*

(3) *For samples that are five or less :*

Leaving one or two the rest of the sample is taken as the *starting sample.*

C. Preparation of the Final Sample ;

The starting sample is put in a mortar and ground loosely so that pellets are broken up as discrete particles in a coarse powder form ; the grinding should not fractionate the particles but merely separate the agglomerates into single particles, large or small, as it may be. Three sieves (ASTM No. 30, 40, 50 approx.) are placed one above the other and the powder sieved ; the portion on the top sieve is rejected ; (A cursory examination under a stereo-microscope of the larger particles in this fraction before rejection would help in diagnosis later). The fractions of the middle and bottoms sieves, as well as that in the ' tails ' (portion that has passed through the bottom-most sieve) are stirred up and a little portion from each of the three are taken), they are mixed thoroughly, and halved ; one half is the '*final sample*' ; the other half should serve as *reserve* in case the final sample is lost in the subsequent processing ; after the slides have been made, the *reserve* may also be rejected. The final sample should not be less than a heaped coffee spoon.

The final sample is boiled in about 2 or 3 ml of chloral hydrate solution over a water bath of boiling water for a few minutes. If the chloral hydrate is too dark or coloured blackish, the powder is allowed to settle, supernatant poured off and fresh quantity of chloral hydrate added and the boiling repeated. In some cases a third boiling may be necessary. When the powder appears to the eye as fairly clarified, the cooking may be considered sufficient. After cooling distilled water is added and the material is shaken thoroughly, allowed to settle and supernatant poured off. This washing is repeated till all the chloral hydrate is washed off.

A dehydration process with alcohol follows, the washing being repeated two or three times to remove all water. Thereafter the same procedure of alcohol : xylol treatment as given for the other samples is followed and the final mounts are made in canada balsam or aroclor.

Diagnostic Keys

(1) *Basis on which developed :*

The most difficult part of the work was the preparation of a satisfactory key. Any key devised for such a purpose as in this work should be based on characteristic evidences in the undigested plant debris present in the faeces. Such characteristics should not only be constant but also fairly specific for a plant. Microscopic characteristics of plants that are normally present in plant powders and serve for diagnosis are the epidermal cell characteristics, trichomes, crystalline or amorphous inclusions, fibres, quantitative indices like the number of ray cell tiers, palisade ratio, vein islet number, epidermal cells per unit area, stomatal number etc., and any other peculiarities. But in this work they were of limited value. The ubiquity of similar anatomical characters in a society of plants growing under similar ecological conditions, the absence of useful characteristic tissues due to digestion inside the animal, the loss of integrity of the tissue systems that could serve for diagnosis, and the severe changes undergone by the tissues and their contents making them unrecognisable, and other such causes lower the value of such characteristics as given above as diagnostic features. Keys based on epidermal tissue and on the mechanical tissues were prepared, but failed to be valid when tested on ' wild ' samples and were therefore rejected.

For the same reason, quantitative values could not be used as a basis for the key although these are very valuable, particularly the ' pallisade ratio ', in identification in intact tissue systems or pure vegetable powders. A single key based on a single factor was also found

to be insufficient as it did not cover all the plants present in the debris. The problems were finally solved by preparing four different keys, keeping one as the ' main key ', and the others as ' supplementary '. The main key is based on the structure, dimension and abundance of the trichomes. The trichomes, which are a constant feature for any plant, were found to be undamaged in the faeces, presented sufficient variation and were easily recognised under the microscope. Of the selected 20 plants given by the workers as forming the chief diet of the wild animals, (as a result of their work with control animals) the main key serves to distinguish 60 to 70 per cent straightaway. Of the remaining, all except the five grasses can be located by combination of the main key and the supplementary keys and by cross-checking. A cross-checking is necessary because the trichomes are neither abundant nor peculiar in these, and the supplementary keys have been based on certain other features of these plants that are present in the debris. The only plant rather difficult to locate quickly is *Wrightia tinctoria*, and the characteristics of this plant has to be specially looked for in the preparations.

A key was prepared for the grasses but failed in application. Identification of grasses are much facilitated by the epidermal peel characteristics. But the debris in the pellets do not show sufficiently large intact epidermal pieces to be useful as basis for a key. For one, there are very few epidermal pieces in the pellets. Secondly, the pieces present are linear in shape rather than quadrangular, rarely more than 40 to 60μ in maximum dimension, so that the cell characteristics are not evident. Although the tiniest bit is sufficient to recognise a piece as ' grass ' it is very difficult to identify the species. The trichomes of the grasses are characteristic of the group as a whole but are inefficient as pointers for the identity of the individual. Therefore a key for the grasses had to be given up. The keys include the features of grasses only to differentiate between them and the other dicots as a whole.

It is also not possible to differentiate between *Acacia catechu* and *A. leucophloea*, although it is very easy to recognise *Acacia* as a genus.

But for these limitations, the key serves to identity about all the plants conclusively. The key has been applied and tested on about a hundred wild sample preparations for its validity. In the beginning there were about ten unknowns, but as work proceeded, four of these were identified up to the species level and two up to genus level. There are as yet three or four that cannot be identified, but it is certain that they are not from among the 80 plants supplied as reference by the workers.

(2) *Method of application of the Keys :*

A microscope equipped with preferably wide-field optics, giving a magnification range of 100X to 200X will be sufficient. A stereomicroscope, camera lucida or drawing apparatus, micrometers, tally counters are accessory equipment.

The most convenient magnification is about 150X. A field of view of about 0·8 mm diameter is available at this magnification. Although 100X magnification is also convenient for most particles in a field, this might require switching over to a higher power often for tinier particles. But a 150X shows up the details of tinier particles also and a change over to a higher power is required only occasionally for confirmation.

This work was done under the following conditions:

Microscope : Leitz Ortholux widefield binocular microscope :
Optics : 12·5X apochromatic objective and 12X aplanatic eyepieces ;
Area of one field of view at 150X : approx. 5 sq mm ;

Total area under a cover-slip covering the debris : approximately 380 sq mm with not less than 500 debris particles ;

No. of fields scanned for each preparation : about 75, i.e. the entire preparation ;

No. of particles normally present per field : 3 or 4 of the larger and 2 or 3 of the smaller.

No. of plants approximately present in a preparation : 6 to 8.

Up to 50% of a field has to be scanned carefully. Representative particles from almost all the plants present will have been located and identified by that time and the rest of the slide may be rapidly scanned for any omission. (The thorough mixing of the powder during the preparation of the slides ensures good distribution of particles, and makes this possible.) The slides are scanned field by field, in row after row, and the plants present identified using the main key based on trichomes. About 60% of the total plants present in a sample would be identified.

The slide is then searched again for pieces of tissues showing the characters given in the other keys for the remaining plants, that is, the epidermis, cuticle, crystals, trichome debris, fruit or seed coat debris etc. An example is given below :

Let us say a piece of cuticle is located, intact with group of epidermal cells. If the piece of cuticle is striated, item 2 under A of Key 3, will identify the plant. In case the cuticle is not striated, then the plant is possibly *Randia dumetorum*. The keys where other characteristics of *Randia* is given may be examined and some cross checking done for confirmation.

A thorough aquaintance with the genuine reference slides, practice with the identification and a judicious use of all the keys help the identification work.

(3) *Precaution to be observed while using the key :*

(*a*) Broken vascular fibres should not be confused with broken trichomes ; the former will show slit pits, greater width, a broad lumen and an irregular blunt tip.

(*b*) Proximity of particles from different origin would appear like a whole tissue of one origin and should not be confused with whole intact tissue. Each particle should be considered as a separate entity, unless actual tissue connection is observed between two particles. It often occurs that two particles from entirely different plants lie so closely together as to appear as one and the identity may be misconstrued. For instance, it is quite likely that the curly long trichomes of *Zizyphus jujuba* is entangled in a group of epidermal cells from *Boswellia serrata*. This might lead the observer to miss the presence of the latter and consider only the former to be present. Or the veinlet skeleton of one leaf may lie cunningly over the lamina of another plant and the two taken together might resemble a third one.

(*c*) Isolated crystals, completely free of tissues are helpful, as their dimension and morphology will denote their origin but when such crystals are seen in association with other tissues, it must be made certain that the crystals are within an intact cell. Only then these will serve for diagnosis. If not, they can completely mislead the observer, as they may merely be superimposed on some tissue to which it does not belong.

(*d*) No key should be applied without having studied the reference slides ; the key cannot be successfully applied by a person without botanical knowledge ; that is, application of the key is not a mechanical job.

(*e*) The keys have been built up from the debris present in the pellets of the ' wild ' samples. Several tissue pieces from the genuine reference plant may not be *exactly similar* to the same tissue pieces in the faecal debris in spite of the same processing done in both cases in the preparation of the material. Therefore the observer need not be puzzled if the same characteristic from the reference slides and the ' wild ' slides are not exactly similar. At times this is confusing enough to feel that the plant is a different one from those in the ' keys ', but actually it is not so. An illustration may make this clear : The cuticles of trichomes are generally intact in the slides made from the genuine plants. But the trichomes of the same plant may lose their cuticle in their passage through the animal. This produces a slight change in the morphology of the trichome, particularly with regard to the walls. In *Butea monosperma*, the multicellular

trichome shows the cross partitions of the basal cells very clearly, when cuticle is intact, if the reference slides are seen. But this is only rarely so in the trichome when it appears in the debris where the cuticle is many times lost. The partition walls of the basal cells become indistinct and only the apical cell wall is clearly seen. A careful scrutiny under higher power would reveal the individual basal cells. Another plant that shows a slight difference is *Terminalia bellerica*. This difference in the clarity of the wall may be due to the strength and amount of lignin in the walls of the trichome. Whatever the reason may be, it is necessary to follow the key closely, even if morphologically and at low power, there appears to be a slight difference from what the key implies and what the debris reveals.

1. KEY BASED ON INTACT TRICHOMES

A_1 **Trichomes without arms :**

B_1 Trichomes unicellular :

1. not over 500 microns in length ; lumen as broad as or broader than the total width of walls at the base ; cuticle, slightly warty .. *Acacia* spp.*
2. over 500 microns in length ; yellowish tinged, narrow, cylindrical and almost solid like a cord ; lumen almost absent ; base showing a conical partition .. *Anogeissus latifolia*
3. up to 500 microns or sometimes slightly more ; spinelike and straight ; cuticle warty ; lumen narrow ; epidermal cells at the base of trichome thickwalled .. *Sapindus emarginatus*

B_2 Trichomes multicellular and uniseriate:

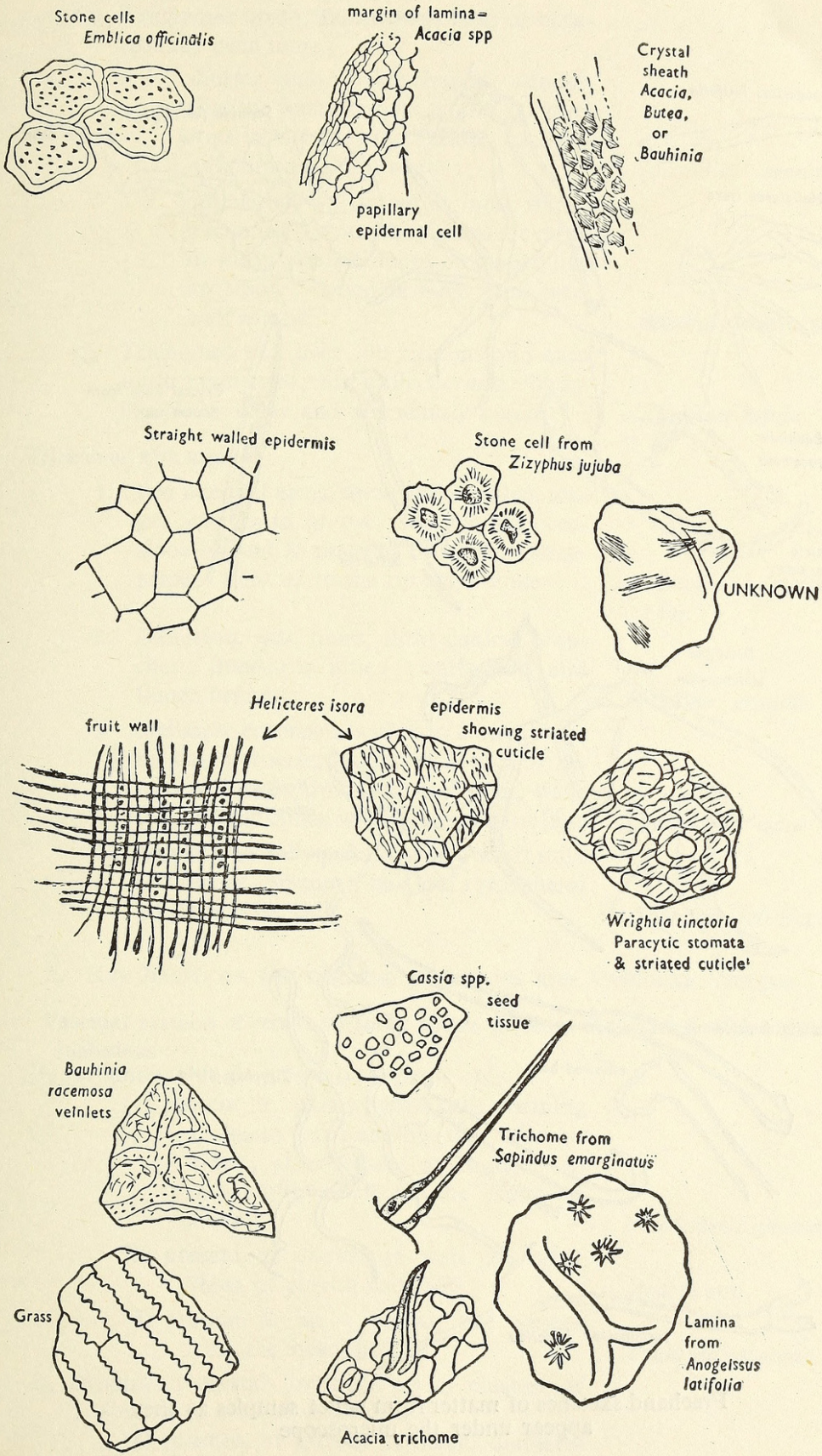
1. under 200 microns, six to seven cells in a short row, stubby and blunt, smaller ones papillose .. *Emblica officinalis**
2. under 200 microns generally, 3 to 4 broad cells of approximately equal height but decreasing width, thin walled, at times covered with loosely fitting cuticle .. *Neuracanthus sphaerostachys**
3. over 500 microns, 2 or 3 basal cells in a short row, apical cell alone very long, at times septate, tips acute .. *Butea monosperma*
4. over 500 microns, single basal cell, and a very long apical cell .. *Terminalia bellerica*

B_3 Trichomes not truly multicellular, but merely septate :

C_1 Up to 300 microns long; cuticle warty ; lumen broader than wall widths ; blunt or rounded tips .. *Bauhinia racemosa**

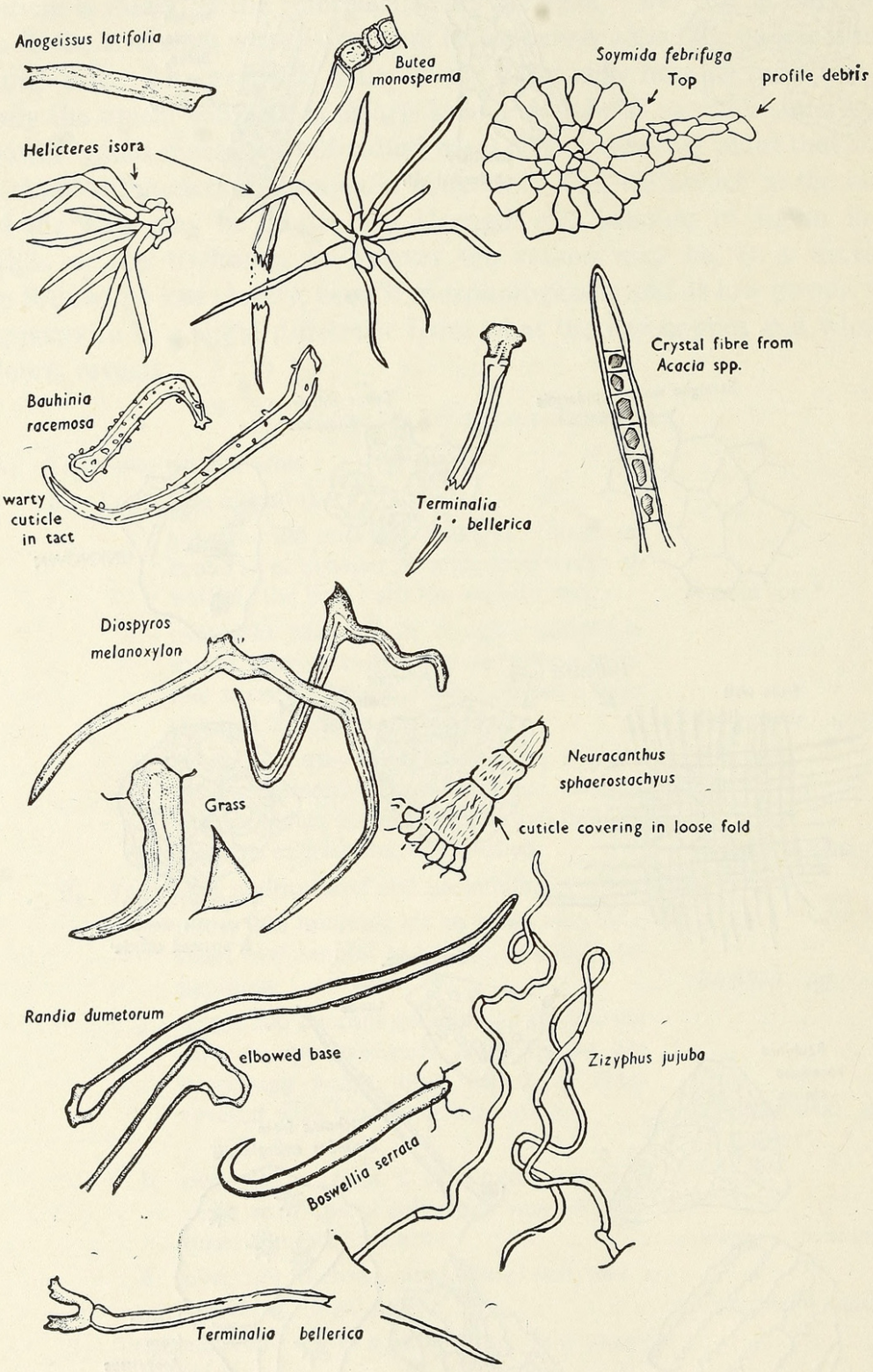
* Trichomes not abundant

Satakopan: *Plant remains*



Freehand sketches of matter from pellet samples as they appear under the microscope

Satakopan : *Plant remains*



Freehand sketches of matter from pellet samples as they appear under the microscope

C_2 Trichomes up to 500 microns long or occasionally even more :

1. shorter ones sickle-shaped, slender, slightly warty cuticle, about 20 microns in width at the middle, base not differentiated, tips acute .. *Boswellia serrata**
2. cuticle not warty, but in loose folds ; trichomes up to 30 microns or more in width ; base bulbous or shaped like an elbow ; lumen broader than total wall widths .. *Randia dumetorum**

C_3 Trichomes well over 500 microns and even up to a mm or so; thin walls, flat and ribbon-like ; very curly and abundantly present .. *Zizyphus jujuba*

A_2 **Trichomes with arms :**

1. two unequal arms, thick and massive, longer arm up to or over 500 microns occasionally, and 35 microns in width, lumen present right up to the tip ; tips acute .. *Diospyros melanoxylon*
2. Dendritic, with blunt, short conical branches ; interwalls pitted ; walls thin and lumen broad .. *Tectona grandis*
3. Stellately branched, with 6 to 8 arms from the base ; arms ranging from 50 to 500 microns in length, tips acute and solid; walls thicker than lumen width, except at the base .. *Helicteres isora*
4. characteristically peltate, with a short stalk and a central group of cells and a peripheral set or ray-like cells .. *Soymida febrifuga*

2. Key Based on Crystalline inclusions and Vascular Systems

A_1 **Vascular systems of veins and shoot apices in close association with crystalline inclusions :**

B_1 Abundant single prismatic crystals only :

(1) present in parenchyma cells forming a sheath surrounding the vascular fibres ; also present at random in mesophyll, often in the form of cross .. *Butea monosperma*

(2) present as above, as well as inside fibres in partitioned tiers .. *Acacia* spp.

(3) present as in 1 above, but absent from the mesophyll .. *Bauhinia racemosa*

B_2 Abundant crystals in the form of clusters of rosettes as well as single ones :

(1) rosettes in greater number, diameter up to 40 microns .. *Boswellia serrata*

(2) rosettes in lesser number, more abundant prisms, large and tabloid .. *Sapindus* spp.

A_2 Vascular systems of veins and shoot apices not in close association with crystals but crystals present in other parts :

B_1 Crystals distributed at random over soft tissue :

(1) xylem vessels pitted with laterally compressed wide pits, appearing scalariform ; large prisms and rosettes present in cortical and pith cells and in mesophyll .. *Emblica officinalis*

(2) xylem vessels wide, pits circular, end-perforation of vessel simple and vessel spurred ; pericyclic fibres present .. *Wrightia tinctoria*

B_2 Crystals only in idioblasts and not at random over the soft tissues :

(1) rosettes over 40 microns in diameter, up to nearly 80 microns ; vascular system showing abundant tracheids and tracheid-fibres .. *Terminalia* spp.

(2) rosettes up to 40 microns or over in diameter, but no tracheids or tracheid-fibres ; vascular fibres present .. *Randia dumetorum*

A_3 Crystals in the form of cystoliths :

(1) clusters in the lamina, very often beneath massive trichomes, epidermal cell walls thick at these places .. *Tectona grandis*

(2) cystoliths in special elongated cells below epidermis, one end broad and other end tapering .. *Neuracanthus sphaerostachys*

3. Key Based on Miscellaneous Debris

A. Cuticle :

1. pieces of cuticle without striations, but with the outline of epidermal cells faintly impressed upon it .. *Randia dumetorum*

2. pieces of cuticle, closely striated, usually intact with the epidermis :

(*a*) cell walls of upper epidermis (no stomata present) very sinuous .. *Diospyros melanoxylon*

(*b*) cell walls of epidermis not sinuous, but wavy to straight :

(*i*) cells of upper epidermis (no stomata) about 10 per 100 microns square .. *Helicteres isora*

(*ii*) cells of upper epidermis (no stomata) about 15 per 100 microns square .. *Wrightia tinctoria*

(*iii*) cells of lower epidermis (stomata present) with anomocytic stomata .. *Soymida febrifuga*

(*iv*) as (*iii*) above, but with paracytic stomata .. *Wrightia tinctoria*